ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5128

Preliminary data on the presence of periosteal growth lines in the mandible of the harbour porpoise, Phocoena phocoena (L.) and their use as age indicators

V. de Buffrenil

Original title: Données préliminaires sur la présence de lignes d'arrêt de croissance périostiques dans la mandibule du marsouin commun, Phocoena phocoena (L.), et leur utilisation comme indicateur de l'âge
In: Can. J. Zool. 60: 2557-2567, 1982

Original language: French

Fisheries & Oceans
LIBRARY
JAN 9 1987
BIBLIOTHÈQUE
Pêches & Océans

Available from:

Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1985

15 typescript pages

90-00544/

TRANSLATION
FROM FRENCH

CTFAS 5128

Preliminary data on the presence of periosteal growth lines in the mandible of the harbour porpoise, Phocoena phocoena (L.), and their use as age indicators

Laboratoire d'Anatomie Comparée, Muséum National d'Histoire Naturelle, [comparative anatomy laboratory, national museum of natural history], 55, rue de Buffon, 75005 Paris, France

and

Equipe "Formations squelettiques," Laboratoire d'Anatomie Comparée de l'Université Paris 7 [skeletal structures team, comparative anatomy laboratory, University of Paris 7], 2, place Jussieu, 75251 Paris, France

Received: February 19, 1982

Introduction

Until now, all age-determination studies of the harbour porpoise (Phocoena phocoena, Linnaeus, 1978) were based on dentinal growth-mark counts. This method was first applied to marine mammals by Scheffer (1950) and Laws (1952) and currently is the most frequently used method of age determination in odontocetes. Nevertheless, the method is known to have serious limitations, which have been reported in several studies of Delphinidae (int al Sergeant 1962; Hui 1978, 1980; Kimura 1980). These limitations arise because the structure of dentinal growth marks is variable and dentinal deposition ceases rather early in life. An age-determination technique that would complement or replace the current one based on dentinal growth-mark counts is urgently needed (see Perrin and Myrick 1980 on the subject).

Bone growth marks have not been described in detail in Phocoena phocoena or other small odontocetes except Delphinus delphis (Kleinenberg and Klevezal 1962; Gurevitch et al 1980); therefore, they have not been put to practical use. Our study shows that sharply defined growth lines exist in the mandibular periosteal bone of the harbour porpoise, and attempts to determine their usefulness as age indicators.

Grue Nielsen (1972) was the first to attempt age determination in the harbour porpoise. He used Meyer's hematoxylin stain to reveal growth marks in dentine, and estimated that these lines were laid down at the rate of one per annum. But, after seven or eight lines are deposited, only osteodentine, which does not lend itself to age determination, continues to be formed. Gaskin and Blair (1977) used non-decalcified thin sections, rather than stained sections, to confirm that dentinal marks are deposited at the rate of one per annum, and were able to count as many as 13 marks in one specimen. Van Utrecht (1978) used the same technique and obtained similar results. Stuart and Morejohn (1980) used dentinal growth marks as a substitute for, rather than an absolute indicator of, individual age in the harbour porpoise. These authors used teeth that had been cut and polished. They reported a maximum of nine dental marks in one of their specimens. Lastly, Grue et al (1980) attempted to make a thorough study of the methods of age determination in the harbour porpoise. Specimens of known minimum age enabled them to reaffirm that dentinal marks are deposited at the rate of one

per annum. Their oldest specimen had nine dental marks. Although Grue et al (1980) wished to compare the usefulness of various calcified tissues (dentine, cement and bone) for determining the age of individuals, they realized that in practice they were restricted to the study of dentinal marks only, because their techniques were not suitable for studying marks in bones and cement.

Materials and Methods

Our biological sample consisted of 18 Phocoena phocoena skulls from the collection of the Muséum National d'Histoire Naturelle in Paris. The sex, origin and method of capture (found stranded, or captured at sea) of these animals are unknown. Therefore, our sample is not representative of natural porpoise populations, and a demographic study of the populations cannot be attempted. The length of all the skulls and mandibles was measured. First, the entire mandible of specimen AC.1923.2294 was sliced into 5 mm sections, and the areas where bone growth lines were most likely to be evident were determined empirically. Then, some bone samples and several teeth from the anterior, middle and posterior regions of the mandible were removed from each skull. The bone samples consisted of 5 mm slices cut transversely from two regions of the mandible: (a) a point one-third of the distance from the anterior edge of the mandible, which corresponds to the middle of the tooth raw, and (b) a point two-thirds of the distance from the anterior edge of the mandible, one or two centimetres posterior to the tooth row (see Figure 1 for positions of samples). The teeth of three specimens were missing and therefore could not be sampled.

In our study, we repeated all methods previously used for the study of dental marks in the porpoise. From each specimen, we took one tooth from the centre of the tooth row (where teeth are usually longest), decalcified it with 3% nitric acid for 48 h, then cut it with a freezing microtome along its longitudinal axis into two series of sections: 10 to 15 µm sections to show marks in cement, and 30 µm sections to show marks in dentine. All sections were stained with Erhlich's hematoxylin for 30 to 50 min, depending on their thickness, and viewed at low magnification under a microscope (method used by Grue Nielsen 1972). The other teeth removed from each specimen were coated with a polyester resin (Stratyl CC.108, Rhône-Poulenc), cut longitudinally into two symmetrical halves, polished and viewed under a microscope in reflected light (method used by Stuart and

Morejohn 1980). Then each half-tooth was glued to a microscope slide and ground on an abrasive disk until 80 to 120 µm sections were obtained (method similar to that used by Gaskin and Blair 1977). These thin sections were examined under a microscope in transmitted natural and polarized light.

Bone samples were decalcified with 3% nitric acid for 24 to 36 h, cut with a freezing microtome into 10 to 15 µm sections, stained in Erhlich's hematoxylin for 45 min, and examined at low magnification under a microscope. This is the standard method for revealing growth lines in bone. Every second piece from the mandible of specimen AC.1923.2294, previously cut up into 5 mm slices, was decalcified, sectioned further and stained; the other pieces were used to obtain 100 µm sections of dry bone (the same method as was used for obtaining thin sections of teeth) which were examined in transmitted natural and polarized light.

In almost all current publications, the term "growth layer groups" designates growth marks in calcified biological tissues (cement, dentine and bone). These marks have a characteristically repetitive structure, and the deposition of each mark requires a uniform period of time. Each growth layer group in the dentine of odontocetes supposedly represents an annual (sometimes biannual) deposit. It consists of two successive layers of dentine (which can be subdivided into finer sub-layers) differing in their opaqueness, their affinity for certain histological dyes, and the outline they make on the surface of teeth that have been sawn and treated with acid (see Myrick 1980). The term "growth layer group" has sufficiently widespread use that it does not need to be translated [when used in French-language texts]. It will be applied here to dentinal lines and used in its abbreviated form, "GLG."

The expression "ligne d'arrêt de croissance," or "LAC," (Castanet 1974) is used widely by French-speaking writers, and refers to thin hematoxylinophilic lines present in bones and cement which are used to determine the age of vertebrates. Numerous English equivalents for this expression, many of them questionable, have been used: growth lines, adhesion lines, and so on.[1] The growth lines* (GLs) of bone and cement are simply lines, not layers, as are the

---

1 For synonyms of "ligne d'arrêt de croissance," see Castanet (1981).

* Throughout this text, "growth line" is used as an equivalent of "ligne d'arrêt de croissance" - Tr.

GLGs in dentine; the use of distinct terms makes differentiation between the two types of growth marks possible.

Results

Growth lines in bone

Histology of the mandible and characteristics of bone GLs

In adult porpoises, thin and stained cross sections of the mandible reveal three layers of bone (Figure 3). The outermost layer is of periosteal origin and is made up of lamellar bone characterized by its regular structure. Osseous lamellae and lines which readily accept hematoxylin stain (the growth lines) appear in thin sections of this tissue (Figure 4). These GLs are laid down when bone growth slows considerably or stops periodically (int al Klevezal 1980; Klevezal and Kleinenberg 1967; Castanent et al 1977); they are standard indicators of ontogenetic age and are intrinsic to bone tissue. The GLs run parallel to the external contour of the periosteal layer. The distance between GLs diminishes from the internal to the peripheral part of the bone (Figure 9). The thickness of osseous lamellae between GLs is uniform (5 to 6 μm); however, when the distance between GLs diminished near the periphery of the bone, the number of lamellae between adjacent GLs also diminished (11 lamellae between the fourth and fifth GLs, and only 6 lamellae between the eighth and ninth GLs in specimen AC.1881.230b; see Figure 4). Particularly in young specimens and in the innermost part of the lamellar bone of older specimens, the GLs may split into two (Figure 9), or two GLs may become partly fused. Such anomalies occur only in limited areas along the length of GLs, and "accessory" or "satellite" GLs produced locally by doubling usually accept less hematoxylin stain than the true GLs. No vascular canal or sign of Haversian remodelling appear in the lamellar periosteal bone of the porpoise mandible. Because of the regular structure of this tissue, thin sections viewed in polarized light show marked anisotropy. Osseous lamellae in the tissue are clearly evident as alternating light and dark layers. The GLs, however, are usually difficult to recognize in polarized light because they appear very similar to lamellae.

A very hematoxylinophilic crenellated line separates the internal lamellar layer from the middle bony layer. The latter consists of fibrous bone, which is isotropic when viewed in polarized light, and richly supplied with vascular canals and secondary Haversian systems (Figure 3): it represents a layer of embryonic bone comparable to the mesosteal zone described by Klevezal and Kleinenberg (1967) in Delphinus delphis. In the harbour porpoise, this embryonic bone remains visible throughout life. The hematoxylinophilic line that separates the embryonic bone from the layer of lamellar periosteal bone is probably a resorption line laid down at birth. A similar "birth line" was described by Hay (1980) in Monodon monoceros.

Another, more regular, resorption line separates the embryonic bone from the innermost layer of lamellar endosteal bone (Figure 3).

Variations in this fundamental three-layer arrangement depend on the stage of ontogenetic development and on the part of the mandible from which the section was taken. In very young specimens that died soon after birth, the lamellar periosteal and endosteal layers are either absent or very thin (in the latter case, the neonatal resorption line is present); their thickness is usually proportional to the length of the mandible. Only spongy bone without a lamellar layer is present in the area anterior to the foramina mentalia. Lastly, in various regions of the mandible, complex processes linked to postnatal morphogenesis resorb bone or prevent it from being laid down, and can lead to the absence of the lamellar periosteal layer.

Growth-line counts

The examination of all stained sections from the mandible of specimen AC.1923.2294 revealed the presence of GLs in all areas posterior to the foramina mentalia, except in bony plates where so much resorption of superficial bone had occurred that embryonic bone reached the surface (Figures 2a and 2b).

The number of GLs diminished at the anterior and posterior edges of the area in which the lines occurred (Figure 5); GLs were most numerous and constant in number in the central third of the mandible, an area located largely behind the tooth row. Within this area the GLs at the base of the lingual surface of the

mandible were easiest to count (no resorption, good structural definition and rare occurrence of double GLs). Whenever a bone sample from this region was available in a specimen, it was used for examining and counting GLs in that specimen.

The results of GL counts are shown in Table 1. Twenty-one perfectly visible GLs (Figure 9), the highest count, were found in the mandible of specimen 1881.230b.

Dentinal GLGs and cement GLs

Structural characteristics of dentinal GLGs

It soon became evident that the three techniques for studying tooth structure were not equally effective. Thin sections viewed in transmitted natural and polarized light revealed much more detail and permitted a more accurate study than did stained sections or cut teeth. Our descriptions and counts of dentinal GLGs therefore are based mainly on the examination of thin sections.

In transmitted natural light, the structure of dentinal GLGs of the harbour porpoise was similar to that already described in detail by previous authors for other odontocetes (Figures 6, 7a and 7b). A neonatal line, a thin and especially translucent layer, appeared in the peripheral part of the dentine. This line separated the very opaque prenatal dentine from the first layer of dentine deposited after birth, which was also very opaque. Postnatal dentine appeared as a succession of alternating translucent and opaque layer; a layer of translucent dentine, together with a layer of opaque dentine, formed one GLG. In the study specimens, GLGs in the crown differed somewhat from those in the root. In the root, less optical contrast existed between the translucent and opaque layers of a single GLG, and GLGs were frequently very poorly defined and indistinct.

In polarized light, layers of translucent dentine appeared anisotropic (the overall reaction was only slight), and opaque layers, isotropic. The position of some anisotropic layers visible in polarized light did not correspond to the translucent layers seen in natural light (Figure 7c). These additional anisotropic layers probably represented the "accessory layers" reported in several odontocetes by Klevezal and Kleinenberg (1967).

No osteodentine deposit was evident in any of the specimens. In one case, however, many small spheres of dentine, similar to those described in Physeter macrocephalus (Boshma 1938), were visible around the pulp cavity.

Growth lines in cement

Except in the teeth of very young specimens, the relatively thick circumradicular layer of cement usually reached 200 to 400 µm. In thin sections, no growth lines were visible in the cement. In 10 to 15 µm stained sections, one or two slightly stained hematoxylinophilic lines were visible in the cement of several specimens.

Dentinal GLG counts

In all specimens, dentinal GLGs were counted in thin sections of crowns examined in transmitted natural light. Accessory layers were eliminated by this counting method. The translucent layer was used to locate the GLGs.

The GLG counts obtained from the various teeth of a specimen were essentially the same (Figures 7a and 7b).

Two main findings emerge from the results shown in Table 1: (i) In our sample, none of the teeth had more than seven clearly identifiable GLGs (Figure 6). The pulp cavities of teeth with seven translucent layers were very narrow (200 to 250 µm), but not completely occluded. Moreover, dentine continued to be deposited for some time after the last visible GLG was laid down, but appeared uniformly translucent and was not arranged in distinct light and dark layers useful for age determination. (ii) The number of dental GLGs of an individual corresponded to the number of GLs present in its mandibular periosteal bone (see, for example, Figures 7a, 7b and 8). This relationship did not exist in old specimens (13 or more GLs), where no dentinal marks could be seen after the sixth or seventh visible GLG (indicated in Table 1 by 7 6 or 7 7).

Discussion

This study shows that growth lines are present in the mandible of the harbour porpoise, and establishes a basic relationship between the number of GLs and the number of dentinal GLGs. No doubt, dental GLGs are laid down at the rate of one per annum; this conclusion is supported by the results of all authors who used these structures to estimate the age of individual porpoises (Grue Nielsen 1972; Gaskin and Blair 1977; Van Utrecht 1978; Grue et al 1980). Therefore, growth lines in bone are probably deposited at a similar rate. Until now, the existence of bone growth lines, which are - or are thought to be - laid down at the rate of one per annum, has been shown in only four species of odontocetes: the sperm whale (Physeter macrocephalus: Laws 1960; Nishiwaki et al 1961), the common dolphin, (Delphinus delphis: Kleinenberg and Klevezal 1962; Gurevitch et al 1980), the beluga (Delphinapterus leucas: Brodie 1969) and the narwhal (Monodon monoceros: Hay 1980). However, Myrick's data (1980), which shows that 11 to 13 osseous lamellae are always present between adjacent GLs (supposedly, these lamellae indicate lunar months), have not been substantiated: our study shows that the number of osseous lamellae laid down in one year decreases with age. This finding agrees with Klevezal's (1980) deductions about the dynamics of bone growth. Comparative studies, not included in our work, would provide more information on this important problem.

In contrast to the observations made by Grue Nielsen (1972) and Grue et al (1980), our study specimens had few or no cement GLs. This scarcity of GLs does not seem to be attributable to cement thickness, which was considerable, as already mentioned. An experimental bias is possible, but unlikely, because we used the most widely accepted technique for revealing GLs, and because this technique was very effective in revealing the GLs in bone (structurally, bone and cement GLs are very similar). It is more likely that the cement GLs of odontocetes are fairly variable and often quite indistinct (see, for example, Kasuya and Brownell 1979, Plates VI and VII). Grue Nielsen (1972) and Grue et al (1980) did not consider them very useful for precise age determination in Phocoena phocoena.

Our work also shows that, in some ways, bone GLs permit more reliable and complete age determination in the porpoise than do dentinal GLGs. Two major considerations support this interpretation. (i) Because GL structure is simple

(hematoxylinophilic lines), little confusion can occur between actual and accessory layers when properly stained sections of appropriate thickness are used (this type of confusion can easily occur when dental GLGs are used). The occasional doubling or partial fusion of GLs is usually easy to detect because accessory lines accept less dye. (ii) In certain regions of the mandible, periosteal bone and accompanying GLGs are deposited in detectable amounts throughout life and do not undergo resorption. They therefore present a continuous record of age from the animal's birth to its death. This is not the case in dentine; for unknown histogenetic reasons, deposits record age during a limited time only. In our study sample, the age of any animal over seven years old could not be determined from the teeth; bone GLs, on the other hand, permitted estimates up to 21 years without any loss in accuracy with increased age. The maximum age of a harbour porpoise, as determined by the dental GLG method, is 13 years (Gaskin and Blair 1977). Our results indicate that these animals have a much longer life span.

When age is determined from GL counts, bone samples should be taken from a certain location only. Our study shows that GLs are most easily seen and counted in the central third of the mandible, behind the tooth row. Grue et al (1980) failed to find GLs in porpoise mandibles - although the lines are clearly visible in this species - probably not so much because their sections were too thick 30μm), as because they took their bone samples from an inappropriate location (the region just behind the symphysis). Kleinenberg and Klevezal (1962) have shown that, in the mandible of Delphinus delphis, bone GLs are easiest to examine in the area just behind the tooth row. The same is true for the porpoise. The similarity is apparently due to special conditions of postnatal growth and morphogenesis of this part of the mandible. On a more general level, it seems obvious that local GL deposition in the periosteal bone, GL number and their unchanging location during ontogenetic development are closely linked to the overall pattern of mandibular growth, which is probably quite similar in all species of small odontocetes. An accurate knowledge of growth patterns will enable scientists to make informed, rather than empirical, choices when taking bone samples.

Age classes between 5 and 12 years were not represented in our study animals. Although this fact is surprising at first, two explanations are possible: (i) the study sample cannot be considered representative of the structure of natural Phocoena phocoena populations because information on sex, origin, and

method of capture is not available; (ii) if most of these porpoises had beached, which is quite possible, the age class distribution in the sample may indicate that juveniles (0 to 4 years) and very old individuals (more than 13 years) are more likely to become stranded than middle-aged individuals. A study of the age classes of stranded cetaceans would provide detailed information on this subject. Lastly, we could hypothesize that the periodicity of GL deposition changes with age. This hypothesis would be difficult to support because no example of such a change exists in the abundant literature on tetrapods.

A comparison of the mandibular lengths of our study specimens and their ages (dental GLGs or bone GLs) suggests that the size-age ratio varies greatly in individual harbour porpoises. The data of Stuart and Morejohn (1980) show considerable individual variability, which is partly sex-linked, in various morphometric parameters. Moreover, Van Utrecht (1978) suggested that individuals from different populations may have unequal growth rates. In our study, this natural variability was probably increased artificially by the probable heterogeneity of our sample. The skull of specimen AC.1881.230b seemed abnormally small (244 mm) for its age (7 dental GLGs; 21 bone GLs). Yet, Stuart and Morejohn (1980) reported a male from the Pacific (UC.B86878) with nine dental GLGs and a skull of very similar length (258 mm) to that of specimen AC.1881.230b.

Our study of the harbour porpoise shows that, provided certain precautions are taken with respect to method, periosteal GL counts can be used to complement the dentinal GLG method of age determination in many odontocetes. These two methods are not mutually exclusive: dental GLGs are useful age indicators in relatively young specimens and are especially convincing when compared to bone GLs; the bone GL method is currently best suited to old specimens. No doubt, one of the most important practical results of using bone GLs for age determination in odontocetes (especially in small species whose dental growth ceases relatively early in life) will be a major upward revision of longevity estimates and, related to this, a re-evaluation of theories on odontocete population dynamics.

Acknowledgments

I would like to thank Professor J Anthony, director of the Laboratoire d'Anatomie Comparée of the Muséum National d'Histoire Naturelle, and D. Robineau,

assistant-lecturer at the same laboratory, who gave me free access to the collection of marine mammals in their charge and permitted me to remove bone and dental samples from porpoise skulls. I also appreciate the helpful advice provided throughout this work by my colleagues J Castanet, A de Ricqlès and M Pascal (Laboratoire d'Anatomie Comparée de l'Université Paris 7).

[See pp 2566 and 2567 of French text for references.]

Figures

Figure 1: Locations from which bone and tooth samples were removed from each specimen: (a) labial view of mandible; (b) lingual view of mandible. Dotted rectangles: bone samples; horizontal lines: tooth samples.

Figure 2: Location of bone growth lines in the mandible: (a) labial view of mandible; (b) lingual view of mandible. Growth lines (GLs) are present in sparsely dotted areas; the densely dotted areas are the best for counting GLs.

Figure 3: The three-layer structure of the mandible. The upper and lower portions of the photograph show a thin section viewed in transmitted natural light; the centre of the photograph shows the same preparation viewed in polarized light; L: Lamellar periosteal bone; E: Endosteal bone; Em: Embryonic bone. The lamellar periosteal and endosteal layers are anisotropic; the embryonic bone is isotropic and highly remodelled.

Figure 4: GLs and osseous lamellae in lamellar periosteal bone. Erhlich's hematoxylin stain. The horizontal lines point to osseous lamellae.

Figure 5: Variation in GL number with location along the mandible of the harbour porpoise (specimen AC.1923.2294).

Figure 6: Growth layer groups (GLGs) in the eighteenth tooth of specimen AC.1881.230b. cé.: cement; c.p.: pulp cavity; d.t.: translucent dentine; é.: enamel; l.n.: neonatal line; numbers from 1 to 7: GLGs in postnatal dentine.

Figure 7: Dental GLGs of specimen AC.1869.380. (a) Seventeenth tooth. Thin sections in transmitted natural light. l.n.: neonatal line; white triangles: GLGs. (b) Second tooth. Thin sections in transmitted natural light. Same abbreviations and symbols as in (a). (c) Same preparation as in (b). Polarized light. Additional GLGs, not visible in natural light, become evident in polarized light.

Figure 8: Lamellar periosteal bone of specimen AC-1869.380. Erhlich's hematoxylin stain. Black triangles: GLs; l.r.n.: neonatal resorption line. The number of GLs is identical to that of GLGs in Figures 5a and 5b.

Figure 9: Neonatal resorption line and GLs in the mandible of specimen AC.1881.230. Erhlich's hematoxylin stain. Same abbreviations and symbols as in Figure 6.

Tables

Table 1: In this study, the following parameters were obtained for all specimens of Phocoena phocoena: skull length, mandibular length, number of dentinal growth layer groups (GLGs), and number of growth lines (GLs) in the mandibular periosteal bone.

Specimen	Length of skull (mm)	Length of mandible (mm)	Dental GLGs	Bone GLs

[For figures, see table on page 2564 of French text.]